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# Flexible Nanoassembly for Sequestering Non-Native Proteins

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A crystal structure of a yeast small heat shock protein reported by Hanazono and colleagues in this issue of *Structure* reveals the versatility of the  $\alpha$ -crystallin domain dimer for building assemblies of different size and symmetry. The domains assemble into a vessel filled with hydrophobic sequence extensions enriched with phenylalanines.

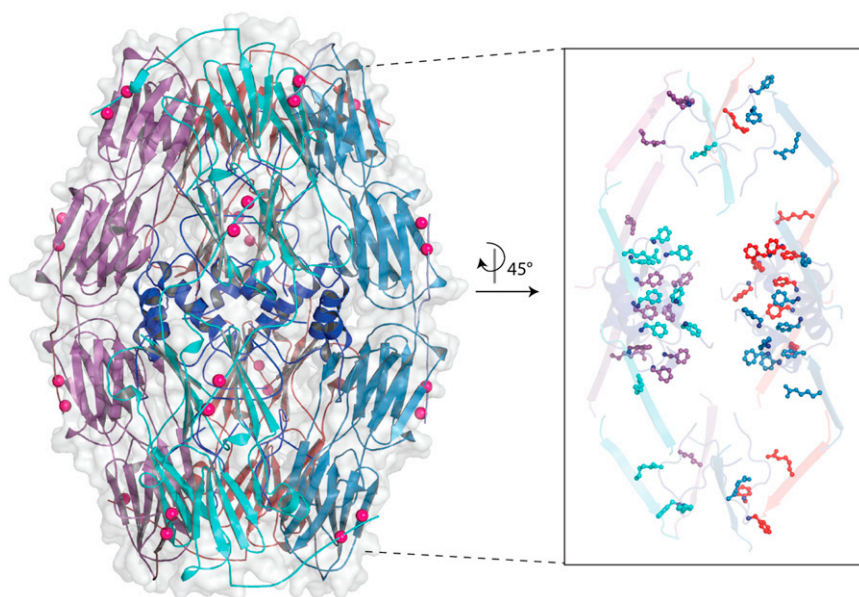
It is a harsh world for the proteome. Living organisms require chaperones to protect cellular functions from aggregating proteins, particularly in times of stress. Among the many chaperone classes, first responders include members of the small heat shock protein (sHsp) family. These are small protein chains that assemble into a wide range of interconverting large oligomers, making their structural characterization difficult. Snapshots of two archaeal sHsp 24-mers, one a hyperthermophile (Protein Data Bank [PDB]: 1SHS), the other an acidophile (PDB: 3VQK), show they take the form of a regular octahedron, whereas a sHsp from the mesophilic wheat forms a less symmetric dodecamer (PDB: 1GME). In this issue of *Structure*, Hanazono et al. (2013) report the crystal structure of stress-inducible SpHsp16.0 from *Schizosaccharomyces pombe* assembled as a 16-mer (PDB: 3W1Z).

sHsp chains fold into a  $\beta$  sheet sandwich, known as the “ $\alpha$ -crystallin domain” in recognition of its first sighting in the vertebrate eye lens protein,  $\alpha$ -crystallin. The domain has a large hairpin loop that is used to form a brick-shaped dimer, with higher assembly driven by flanking sequence extensions. C-terminal I-X-I/V sequence motifs extend from both ends of the dimer and insert into the side pockets of  $\beta$  sandwiches from adjacent dimers. Conformational flexibility in the linker between the domain and its C-terminal strap allows formation of a wide range of nano-assemblies. For example, the archaeal sHsps comprise 24 identical monomers that assemble into spherical shells with the 432 symmetry of a regular octahedron; each

dimer lies along an edge and a single linker conformation allows four dimers to assemble around each of the six vertices using the C-strap into sandwich pocket interaction. However, in wheat sHsp, two linker conformations enable formation of a less symmetric dihedral hexamer of dimers; the C-strap into pocket interaction directs a ring of dimers around a 3-fold axis using one linker conformation, while another links side-by-side dimers about a 2-fold axis, weaving two hexameric rings together. All sHsps have highly hydrophobic N-terminal extensions of great sequence diversity (Basha et al., 2012). The archaeal sHsps, which can be assembled without N-terminal extensions, have the C-terminal straps on the outside of spherical shells that enclose much of the disordered N-terminal extensions. Interestingly, when an archaeal N-terminal region was engineered to be longer, the assembly doubled in size to a 48-mer (PDB: 4ELD) as a result of the linker now taking up alternative conformations (McHaourab et al., 2012). In the wheat sHsp, 6 of the 12 N-terminal extensions were observed to be contributing to the final assembly forming helical pairings between the rings, and even a loose knot, inside the double disc. The structural biological role of the N-terminal extensions is unclear.

The new structure for yeast SpHsp16 is an octomer of dimers arranged with dihedral (422) symmetry. The assembly is organized as AB and DC dimers with the linker that connects the C-terminal I-X-I motif to the  $\alpha$ -crystallin domain in two conformations. The assembly can be described as a lens-like spheroid, with two dimers lying end-to-end along four

lines of longitude (shown in uniform color in Figure 1). At each of the poles, the C-terminal I-X-I motifs (from chains A and D) bind into pockets (formed from chains D and A respectively) about the 4-fold axis, much the same as what occurs around the vertices in the archaeal octahedrons. The partner chains (B and C) in the dimers use an alternative linker conformation to allow their C-terminal extensions to make a 2-fold interaction across the equator linking the two hemispheroids together. In this way, the same I-X-I (Ile shown as hot pink balls in Figure 1) into pockets interaction is used to construct the four (anti-parallel) staves of the spheroid and lock them together around the poles. This makes a precariously flexible assembly, equivalent to removing four edges from around an octahedron, but it is made more rigid by four copies of the N-terminal extension of chain B in the assembly, which is completely defined in the crystal structure and by the less well resolved C chains (colored dark blue, Figure 1, left). These additional interactions between N-terminal extensions make connections between staves and across the equatorial zone, involving an array of intrachain and interdimer phenylalanine and arginine interactions (Figure 1, right). The arrangement leaves much of the hydrophobic character of the N-terminal region unsatisfied inside the equatorial zone of the spheroid. This new sHsp structure provides evidence of a vessel assembled using straps exposed to the outside world, while the bulk of the hydrophobic N-terminal extensions are encapsulated within. If this structure resembles the inactivated state of the chaperone, then



**Figure 1. The Shape of a Small Heat Shock Protein Hexadecamer**

Left: Yeast SpHsp16.0 assembles as a 16-mer with the  $\beta$  sheet sandwich of the  $\alpha$ -crystallin domain shown in ribbon and the C-terminal I-X-I motif depicted as hot pink balls. The shape is spheroid with four domains (in uniform color) forming four lines of latitude, with chains A and D around the poles and chains B and C around the equator. The transparent surface of the  $\alpha$ -crystallin domains are shown in light gray. All the resolved N-terminal extensions, shown in dark blue strand and alpha helices shown as ribbon, are encapsulated within the vessel.

Right: A more detailed view of the interior showing the resolved N-terminal phenylalanines clustering around the equatorial zone and the location of the superfamily conserved arginine on the dimer interface  $\beta$  strand.

a flexible assembly mechanism would allow the easy exposure of the hydrophobic N-terminal extensions for binding denatured proteins, with the C-strap into pocket interaction driving reassembly into some shape or other.

Does the structure shed light on the animal sHsps that play protective roles under stress conditions, making them important for human health? Transfer of insights gained from the yeast, plant, and archaeal sHsp X-ray structures to metazoans is hampered by the fact that, although the C-terminal strap into pockets interaction is conserved, the animal dimer interface is different. There is, however, an enigmatic kingdom-wide conserved arginine that is always ion-paired at dimer interfaces. It is mutated in several inherited human diseases, including childhood cataract. The equivalent residue in SpHsp16 is Arg 102, which

is ion paired at the dimer interface with Glu 92 and facing toward the interior of the spheroid in the vicinity of the encapsulated N-terminal phenylalanine-arginine interactions (Figure 1 right), as it is in the other sHsp assembly structures. In snapshots of several mammalian  $\alpha$ -crystallin domain dimers, the equivalent interface ion pair is found at either end of a deep groove, which we have argued is a candidate-binding site for unfolded polypeptide chain (Clark et al., 2011).

A formidable challenge is to interpret these solid-state structures in the light of their extremely dynamic solution behavior (Baldwin et al., 2011; Delbecq et al., 2013). The archaeal sHsp structures adapted for extreme conditions form thermostable structures resembling platonic solids without requiring the hydrophobic N-terminal extension for assembly. The mesophilic structures form less sym-

metric assemblies with involvement of their hydrophobic N-terminal sequences. The new SpHsp16 assembly defines an interior space containing ordered and unresolved N-terminal extensions rich in phenylalanines. In human  $\alpha$ B-crystallin, which is upregulated in muscle under stress, the most enriched residue (comprising 15%) of the N-terminal extension sequence is phenylalanine. The new SpHsp16 structure supports the view that sHsp assemblies can use hydrophobic N-terminal regions to facilitate dynamic assembly and provide a dynamic hydrophobic environment to stabilize denatured proteins. This new structure of a 16-mer, along with previously resolved tetrahedral (point group 23) and dihedral (point group 32) 12-mers and octahedral (432) 24- and 48-mers, provides ideas of how a single chain, like  $\alpha$ B-crystallin, might assemble into a wide range of interconverting oligomers (Baldwin et al., 2011) fit for the purpose of sequestering and maintaining denatured proteins in solution as well as ensuring eye lens transparency (Slingsby et al., 2013).

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